41. The process according to claim 2 comprising culturing the microorganism in a culture medium for culturing a microorganism in which the concentrations of sodium ions, magnesium ions, and calcium ions in the culture medium are in the range of 5 to 40 mM, 1 to 6 mM, and 1 to 9 mM, respectively.

C11 Cont

42. The process according to claim 6 wherein said unsaturated fatty acid is selected from the group consisting of γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, eicosapentaenoic acid, 5,8,11-eicosatrienoic acid, 6,9-octadecadienoic acid, and 8,11-eicosadienoic acid.--

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REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application, in light of the following remarks and pursuant to 37 C.F.R. § 1.112, are respectfully requested. Support for the amendment to claim 1 may be found, at the very least, on page 9, line 30, to page 10, line 7, of the specification as filed. Support for the amendments to claims 7, 12 and 30 (inserting the chemical name for Mead acid, i.e. 5,8,11-eicosatrienoic acid) is found in the general knowledge of one of skill in the art at the time the application was filed. One of skill in the art would be aware of the chemical name for Mead acid. See, for example, the attached abstract (Retterstol et al, *Biochim. Biophys. Acta 1259(1)*:82-88 (1995)) (Exhibit A) which is for an article published prior to the earliest filing date of the above-identified application. The remaining amendments to the

claims are for just clarifying purposes. No new matter has been added by the present amendment.

Rejection of Claims 1-3, 6-12 and 14-38 Under 35 U.S.C. § 112, Second Paragraph

Claims 1-3, 6-12 and 14-38 have been rejected under 35 U.S.C. § 112, second paragraph, for purportedly being indefinite. For at least all of the reasons set forth below, withdrawal of this rejection is believed to be in order.

According to the Examiner, claim 1 is indefinite for a variety of reasons. Applicants have amended claim 1 to clarify the claim, and provide the following remarks.

To fully explain the present invention, two cases must be considered. In the first case, the production of unsaturated fatty acids increases positively depending on the amount of microbial cells present in the culture. In other words, by increasing the amount of microbial cells present in the culture, one can increase the production of unsaturated fatty acids. In the second case, the production of unsaturated fatty acids does not positively relate to the amount of microbial cells present in the culture. In this case, it is not necessary to increase the amount of microbial cells to enhance the production of unsaturated fatty acids.

Regarding the first case, to increase the amount of the microbial cells of a microorganism belonging to the subgenus *Mortierella*, an increase of the proportion of the pulp form is necessary. In this regard, when the claimed microorganism is cultured in a liquid medium, a cultured microbial mass is a mixture of the pellet form and the pulp form. If the ratio of the pulp form is increased, the viscosity of the medium increases, and

therefore oxygen supply decreases. As a result, an increase of the microbial cells stops, and therefore high productivity of the unsaturated fatty acids cannot be obtained.

Incidently, the pellet form of the microbial cells has a relatively thick cell wall, and therefore oxygen supply into the microbial cells is inhibited by the cell wall. On the other hand, the pulp form of the microbial cells has a relatively thin cell wall, and therefore the oxygen supply into the microbial cells is not inhibited by the cell wall. As explained above, in the second case, an increase of the microbial cells does not enhance the productivity of the unsaturated fatty acids. Rather, the productivity of the unsaturated fatty acids positively depends on the oxygen supply into the microbial cells. Therefore, an increase of the ratio of the pulp form results in an enhancement of the productivity of the unsaturated fatty acids.

Therefore, the present invention covers an artificial increase of the ratio of the pellet form and an artificial increase of the ratio of the pulp form. According to the present invention, an increase in the amount of phosphate anions added to a culture medium increases the ratio of the pulp form; and an increase in the amount of sodium, potassium and/or magnesium cations added to a culture medium increases the ratio of the pellet form. In other words, according to the present invention, the ratio (or balance) of the pellet form and the pulp form is controlled by adjusting concentrations of ions added to a culture medium.

Regarding the metes and bound of the subgenus *Mortierella*, it is clear from the Amano et al reference, already submitted, what is encompassed by the subgenus *Mortierella*.

Finally, as explained above, the claimed invention is drawn to a process for culturing a microorganism characterized by controlling the ratio of the pellet form and the pulp form of the microbial cells by adjusting the concentrations of ions added to a culture medium. The advantage of the process of the present invention is that the production of an unsaturated fatty acid by the microorganism is enhanced. Claim 1 is drawn to a process for culturing a microorganism, not a process of producing unsaturated fatty acids (see claims 8 and 9 for such a claim). Therefore it is not believed that a recovery step is necessary in claim 1.

With regard to claim 1 being purportedly incomplete for not providing antecedent basis for "calcium ions," this phrase has been deleted from claims 2-3 and 7.

Claims 21 and 22 are purportedly vague for being apparently limited to phosphate, sodium, magnesium and calcium ions. Claims 21 and 22 have been amended to recite that the culture medium comprises these ions, thereby clarifying the claims.

According to the Examiner, claims 7, 12 and 30 are confusing with respect to the language "Mead acid." Claims 7, 12 and 30 have been amended to insert the chemical name for this compound.

Claim 29 has been amended to remove the parenthetical recitations of the formulas, thereby clarifying the claims.

Claims 23 and 38 have been canceled, without prejudice or disclaimer to the subject matter disclosed therein. Thus, the rejection of these claims is moot.

Finally, claim 35 is purportedly vague for encompassing an improper Markush group. Claim 35 has been amended to clarify the claim and to remove the phrase "together with any of the above treatments."

In light of the above remarks, applicants respectfully request withdrawal of these rejections under 35 U.S.C. § 112, second paragraph.

Rejection of Claims 21-22 and 38 Under 35 U.S.C. §§ 102(b) or 103(a)

Claims 21-22 and 38 have been rejected under 35 U.S.C. §§ 102(b) or 103(a), for purportedly being anticipated by or alternatively obvious over Sigma Catalog. For at least all of the reasons set forth below, withdrawal of this rejection is believed to be in order.

The present invention is directed to a culture medium for culturing microorganisms belonging to the subgenus *Mortierella*. In contrast, the Sigma Catalog discloses a medium for culturing insect cells (specifically *Drosophila melanogaster*). The Sigma Catalog does not disclose or suggest a medium for culturing fungi, as the Examiner purports.

It is well known to one of skill in the art that optimum medium condition is dependent on the organism to be cultured. The microorganisms belonging to the subgenus *Mortierella* are completely different from insect cells. Therefore, the Sigma Catalog does not describe or suggest a medium suitable for culturing a microorganism from the subgenus *Mortierella*.

In light of these remarks, applicants respectfully request withdrawal of this rejection under 35 U.S.C. §§ 102(b) or 103(a).

Rejection of Claims 1-3, 6-12 and 14-38 Under 35 U.S.C. § 103(a)

Claims 1-3, 6-12 and 14-38 have been rejected under 35 U.S.C. § 103(a) for purportedly being unpatentable over Suzuki et al taken with Manoh et al and Yamaguchi et al.

Suzuki et al discloses a process for producing fatty acid in a medium. Suzuki et al does not disclose or suggest adjusting the kind and concentrations of components of a medium suitable for culturing microorganisms of the subgenus *Mortierella*.

Manoh et al disclose a process for culturing the fungus *Cummingmella elegans*. Manoh et al does not disclose or suggest culturing a microorganism belonging to the subgenus *Mortierella*.

Yamaguchi et al disclose a method for culturing *Aspergillus ciavatus*. Yamaguchi et al does not disclose or suggest culturing a microorganism belonging to the subgenus *Mortierella*.

None of the references cited by the Examiner disclose or suggest a culture medium for culturing a microorganism belonging to the subgenus *Mortierella*. Thus, even if one were to take the disclosure of Suzuki et al to adjust the components of the culture mediums disclosed by either Manoh et al or Yamaguchi et al so that they produce more fatty acids, one would not arrive at the culture mediums of the present invention since, as is known by one of skill in the art, the optimum medium conditions are dependent on the organism to be cultured. The optimum medium conditions for *Cummingmella elegans* or *Aspergillus ciavatus* are quite different from the optimum medium conditions for a microorganism selected from the subgenus *Mortierella*. This is especially true since an important aspect of

the present invention is controlling the proportion of the mycelia in the pulp form, as compared to the proportion of the mycelia in the pellet form. As discussed in more detail above, adjusting the proportions of the pulp and pellet forms of the microorganism from the subgenus *Mortierella* is important for enhancing the production of unsaturated fatty acids. None of the cited references disclose or suggest adjusting the proportions of the pulp and pellet forms, by adjusting the contents of the culture medium, as a means to enhance production of unsaturated fatty acids. Therefore, even if the disclosures of Suzuki et al, Manoh et al and Yamaguchi et al were taken together, one would not arrive at the culture mediums and processes claimed in the present invention.

For at least all of the reasons set forth below, withdrawal of this rejection under 35 U.S.C. § 103(a) is respectfully requested.

CONCLUSION

From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order and such action is earnestly solicited.

In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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Attachment to Amendment and Reply dated October 15, 2001

Marked-up Claims 1-3, 7, 11-12, 18, 21-22, 28-30, 33 and 35

1. (Twice Amended) A process for culturing a microorganism belonging to the subgenus *Mortierella*, wherein the [balance of the pellet form and] proportion of the mycelia in the pulp form [of the microorganism] is [controlled] increased by [adjusting] increasing the concentrations of phosphate ions[, potassium ions, sodium ions, magnesium unsaturated fatty acids by the microorganism is enhanced] in the culture medium, thereby enhancing the production of an unsaturated fatty acid by the microorganism.

2. (Twice Amended) The process according to claim 1 comprising culturing the microorganism in a culture medium for culturing a microorganism in which the concentrations of phosphate ions[, potassium ions, sodium ions, magnesium ions, and calcium ions] in the culture medium are in the range of 5 to 60 mM[, 5 to 60 mM, 2 to 50 mM, 0.5 to 9 mM, and 0.5 to 12 mM, respectively].

3. (Twice Amended) The process according to claim 2 comprising culturing the microorganism in a culture medium for culturing a microorganism in which the concentrations of phosphate ions[, potassium ions, sodium ions, magnesium ions, and calcium ions] in the culture medium are in the range of 10 to 45 mM[, 5 to 40 mM, 1 to 6 mM, and 1 to 9 mM, respectively].

Attachment to Amendment and Reply dated October 15, 2001

Marked-up Claims 1-3, 7, 11-12, 18, 21-22, 28-30, 33 and 35

7. (Amended) The process according to claim 6 wherein said unsaturated fatty acid is selected from the group consisting of γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, eicosapentaenoic acid, [Mead acid] 5,8,11-eicosatrienoic acid, 6,9-octadecadienoic acid, and 8,11-eicosadienoic acid.

11. (Twice Amended) The process for production according to claim 8 wherein said ions are provided by a combination of potassium dihydrogen phosphate [(KH_2PO_4)], anhydrous sodium sulfate [(Na_2SO_4)], magnesium chloride hexahydrate [($MgCl_2 \cdot 6H_2O$)] and calcium chloride dihydrate [($CaCl_2 \cdot 2H_2O$)].

12. (Twice Amended) The process for production according to claim 8 wherein said unsaturated fatty acids are arachidonic acid, γ-linolenic acid, dihomo-γ-linolenic acid, [Mead acid] 5,8,11-eicosatrienoic acid and/or eicosapentaenoic acid.

18. (Twice Amended) The process for production according to claim 17 wherein the processing of said defatted soybeans, or non-defatted soy beans is by heat treatment; acid treatment; alkali treatment; enzyme treatment; chemical modification; denaturation, renaturation or combination thereof, by chemical processing, physical processing, or combination thereof[, together with any of the above treatments]; removal of a portion of the components with water [solvents], organic solvents, or combination thereof; removal of

Attachment to Amendment and Reply dated October 15, 2001

Marked-up Claims 1-3, 7, 11-12, 18, 21-22, 28-30, 33 and 35

a portion of the components by filtration, centrifugation or combination thereof; freezing; crushing; drying; sifting, or combinations thereof.

21. (Twice Amended) A culture medium for culturing a microorganism belonging to the subgenus *Mortierella* [in which the concentrations of] comprising phosphate ions, potassium ions, sodium ions, magnesium ions, and calcium ions [are] in concentrations in the range of 5 to 60 mM, 5 to 60 mM, 2 to 50 mM, 0.5 to 9 mM, and 0.5 to 12 mM, respectively, and wherein said culture medium comprises a nitrogen source and a carbon source.

22. (Twice Amended) A culture medium for culturing a microorganism belonging to the subgenus *Mortierella* [in which the concentrations of] comprising phosphate ions, potassium ions, sodium ions, magnesium ions, and calcium ions [are] in concentrations in the range of 10 to 45 mM, 10 to 45 mM, 5 to 40 mM, 1 to 6 mM, and 1 to 9 mM, respectively, and wherein said culture medium comprises a nitrogen source and a carbon source.

28. (Amended) The process for production according to claim 9 wherein said phosphate ions are provided by at least one salt selected from the group consisting of dipotassium hydrogen phosphate, potassium dihydrogen [phosphsphase] phosphate,

Attachment to Amendment and Reply dated October 15, 2001

Marked-up Claims 1-3, 7, 11-12, 18, 21-22, 28-30, 33 and 35

disodium hydrogen phosphate and sodium dihydrogen phosphate; said potassium ions are provided by at least one salt selected from the group consisting of dipotassium hydrogen phosphate, potassium dihydrogen phosphate and potassium chloride; said sodium ions are provided by at least one [sale] salt selected from the group consisting of disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride and sodium sulfate; said magnesium ions are provided by magnesium chloride and/or magnesium sulfate; and said calcium ions are provided by calcium chloride and/or calcium carbonate.

29. (Amended) The process for production according to claim 9, wherein said ions are provided by a combination of potassium dihydrogen phosphate [(KH_2PO_4)], anhydrous sodium sulfate [(Na_2SO_4)], magnesium chloride hexahydrate [($MgCl_2 \cdot 6H_20$)] and calcium chloride dihydrate [($CaCl_2 \cdot 2H_2O$)].

30. (Amended) The process for production according to claim 9, wherein said unsaturated fatty acids are arachidonic acid, γ-linolenic acid, dihomo-γ-linolenic acid, [Mead acid] 5,8,11-eicosatrienoic acid and/or eicosapentaenoic acid.

33. (Amended) The process for production according to claim 32, wherein the nitrogen source derived from soy [beams] beans has a nitrogen content of at least 2% wt with respect to the total components except for water.

Attachment to Amendment and Reply dated October 15, 2001

Marked-up Claims 1-3, 7, 11-12, 18, 21-22, 28-30, 33 and 35

35. (Amended) The process for production according to claim 34, wherein the processing of said defatted soy beans, or non-defatted soy beans, is by heat treatment; acid treatment; alkali treatment; enzyme treatment; chemical modification; denaturation, renaturation or combination thereof, by chemical processing, physical processing or combination thereof[, together with any of the above treatments]; removal of a portion of the components with water [solvents], organic solvents, or combinations thereof; removal of a portion of the components by filtration, [cetrifugation] centrifugation, or combinations thereof; freezing; crushing; drying; sifting, or combinations thereof.